2. (Twice Amended) A compound as claimed in claim 1, wherein the compound further comprises covalently bonded carbohydrates.

3. (Twice Amended) A compound as claimed in claim 1, wherein [the] at least one antigen binding region comprises a variable domain of a heavy antibody chain and a variable domain of a light antibody chain (sFv fragment).

29. (Twice Amended) A compound as claimed in claim 10, which [undergoes] has undergone secretory expression in *Hansenula polymorpha*.

30. (Amended) A compound as claimed in claim 1, wherein [the] at least one antigen binding region and at least one prodrug-activating enzyme form an sFv-β-lactamase fusion protein.

REMARKS

I. Status of Application

Claims 1-33 are pending, with claims 14-22 being withdrawn from consideration. In the Office Action dated August 7, 2000, the Examiner withdrew the previous objection to the specification and the previous rejections under 35 U.S.C. §101 and §112, as well as the previous obviousness-type double patenting rejection. However, the Examiner maintained the objection under 37 C.F.R. §1.75(c) and rejection under 35 U.S.C. §112, second paragraph, of claim 2; the rejection of claims 3 and 30 under 35 U.S.C. §112, second paragraph; and the prior art rejections of claims 1-13 and 25-33 under 35 U.S.C.

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§103(a). Additionally, the Examiner imposed a new rejection of claim 30 under 35 U.S.C. §112, first paragraph.

At the outset, Applicants thank the Examiner for the helpful comments made during a telephone interview with Applicants' undersigned representative on September 15, 2000, the substance of which is summarized in an Interview Summary of the same date. During the interview, the Examiner withdrew the newly imposed 35 U.S.C. §112, first paragraph (new matter) rejection of claim 30, which was set forth on page 3 of the Office Action dated August 7, 2000.

By this amendment, Applicants propose to amend claims 1-3 and 29-30. Support for these amendments may be found in the specification and claims as originally filed. In particular, the amendments of claims 1, 3, and 30 are supported by the specification on pages 5-6. The amendments of claims 2 and 29 are supported by the claims as originally filed. Reconsideration of claims 1-13 and 25-33 is respectfully requested in view of these amendments and in light of the following comments.

II. Objection under 37 C.F.R. §1.75(c) and Rejection under 35 U.S.C. §112 of Claim 2

The Examiner objected to claim 2 under 37 C.F.R. §1.75(c) as being of improper dependent form for failing to further limit the subject matter of independent claim 1. The Examiner's position is that claim 2's recitation of carbohydrates fails to further limit the protein of independent claim 1. The Examiner further rejected claim 2, along with claims dependent thereon, under the second paragraph of 35 U.S.C. §112, as allegedly confusing as to what is meant by the phrase "comprises covalently bonded carbohydrates."

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The Examiner suggests that the word --further-- be inserted before "comprises" in claim 2 in order to overcome the objection and rejection of these claims. Applicants have amended claim 2 in accordance with the Examiner's suggestion. Applicants therefore request that the objection and rejection of claim 2 be withdrawn.

III. Rejection under 35 U.S.C. §112, second paragraph, of claims 3 and 30

The Examiner maintained the indefiniteness rejection of claim 3 and 30 as being inconsistent with independent claim 1. The Examiner alleges that the sFv fragment recited in claims 3 and 30 is inconsistent with the limitation in independent claim 1 that the antigen binding region has a bivalent or multivalent structure.

During the telephone interview with the Examiner on September 15, 2000, the Examiner maintained his position that claims 3 and 30 do not indicate the concept of two or more antigen binding regions, which would be consistent with the "bivalent or multivalent structure" limitation of claim 1. Accordingly, Applicants have amended claim 1 to recite "at least one antigen binding region" and to recite that the "compound" of claim 1 has a bivalent or multivalent structure. Support for these amendments appears in the paragraph bridging pages 5 and 6 of the specification. Applicants have also amended claims 3 and 30 to change "the antigen binding region" to --at least one antigen binding region-- to be consistent with the amendment of claim 1.

Applicants submit that these amendments remove the inconsistency mentioned by the examiner in the Office Action with respect to the sFv fragment and respectfully request withdrawal of the rejection of claims 3 and 30.

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IV. Rejection under 35 U.S.C. §112, first paragraph, of claim 30

The Examiner imposed a new matter rejection of claim 30, arguing that the subject matter of claim 30 is not supported by the originally filed disclosure. During the above-mentioned telephone interview on September 15, 2000, the Examiner agreed that this rejection was improper and was withdrawn. Therefore, no response to this rejection is needed.

V. Rejections under 35 U.S.C. §103(a)

The Examiner maintained the four obviousness rejections over Bosslet et al. (*Brit. J. Cancer*, 1992) or Seemann et al. (EP 501,215) (hereinafter referred to collectively as "the primary references") in view of Huston et al. (U.S. Pat. No. 5,132,405 and *Methods in Enzymology,* 1992) (hereinafter referred to collectively as "Huston" and specifically as "Huston patent" and "Huston article"), and as necessary Bosslet et al. (EP 040,097) and Eaton et al. (EP 392,745), and further secondary references.

The Examiner's rejections appreciate that the primary references disclose a fusion protein comprising a Fab, a linker, and a human β-glucuronidase. The Examiner's rejections further appreciate that this fusion protein differs from the claimed compound by comprising a Fab (composed of H and C chains) instead of comprising an "antigen binding region . . . composed of a single polypeptide chain," *e.g.,* a sFv. The Examiner's rejections rely on Huston for teaching this element of the claims.

The Examiner has considered Applicants' previous arguments in response to these rejections, but has not been persuaded. The Examiner's position is that sFv and Fab constructs are known to be functional equivalents in the art of immunochemistry, as

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evidenced by the example in the Huston patent in col. 19, as well as the Huston article on page 48. The Examiner also states that Applicants' arguments concerning lack of predictability and stability are not persuasive in view of Huston's overall teaching of sFv's binding properties and increased stability. The Examiner particularly points to the Huston article's teachings in pages 68 (lines 1-5) and 87. Additionally, The Examiner is unconvinced that a person of ordinary skill in the art would not have expected an sFv and pro-drug activating enzyme fusion to result in a non-functional sFv domain. The Examiner cites the Huston article, pages 82-83 and 87, as well as the Huston patent's claims to a fusion construct of an sFv and an effector molecule as evidence that one of ordinary skill in the art would reasonably expect fusion proteins to work without loss of functionality or stability.

Applicants respectfully traverse the obviousness rejections for the reasons set forth in their previous response, incorporated herein by reference, and for the additional reasons set forth below.

A. The Prior Art Does Not Provide Reasonable Expectation that Proposed Modification Will Succeed

Applicants submit that the combination of the primary references with Huston is improper, particularly because the prior art does not provide evidence of a reasonable expectation of success in binding a single polypeptide chain antigen binding region, such as a sFv fragment, with a prodrug-activating enzyme. First, the teachings of Huston, which provide only limited examples of fusion partners, should not be generalized to all fusion proteins, such as prodrug-activating enzymes, because of the very different behavior of these enzymes. Secondly, it is not at all clear whether the

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linker technology presented in Huston would be applicable for fusing an sFv molecule to any fusion partner, such as a prodrug-activating enzyme, and in fact, there is evidence that Huston's linker technology would not work in the present invention. Furthermore, none of the various secondary references cited by the Examiner cure these deficiencies.

1. Huston's limited teachings of fusion partners cannot be generalized to a prodrug-activating enzyme

Huston does not disclose a sFv molecule having antitumor specificity, *i.e.,* linked to a prodrug-activating enzyme. The example in Huston refers to an anti-digoxin sFv molecule. (*See* Huston article, page 71, and examples in Huston patent.) It is well known that digoxin works quite well when used as an antigen whereas tumor antigens are more difficult to work with and behave differently than other antigens. Therefore, a person of ordinary skill in the art at the time of the claimed invention would not have expected to be able to substitute the sFv fragment of Huston for the Fab fragment of the primary references with a reasonable expectation of success in order to arrive at the claimed compounds.

Furthermore, it is true that several fusion partners have been mentioned in Huston. On page 57 of the Huston article, a *Pseudomonas* exotoxin is mentioned, along with fragment B of staphylococcal protein A. On page 59 of the Huston article, a diphtheria toxin fragment is mentioned. However, in all cases these fusion proteins have been tested for their binding activities concerning the sFv portions of the molecule. No evidence is presented whether the activity of the fusion partner itself, not being a sFv, would be sufficient to activate prodrugs. A person of ordinary skill in the art, therefore, would not have been motivated to substitute the sFv fragments of Huston for

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the Fab fragments in the primary references because there would not have been a reasonable expectation of success in fusing a sFv to a prodrug-activating enzyme.

Finally, as the Examiner as pointed out, the claims of the Huston patent recite fusion proteins comprising a third amino acid sequence having biological activity. While this portion of the claims is broadly supported in the paragraph bridging columns 3 and 4 of the Huston patent, it does not in any way suggest that a person of ordinary skill in the art would have had a reasonable expectation of success in arriving at the claimed invention.

Thus, since very limited, if any, data on the activity of the fusion partner linked to a sFv molecule is given by Huston, a person of ordinary skill in the art would not appreciate that full functionality of both protein partners could be achieved by linking a sFv fragment with at least one prodrug-activating enzyme.

2. Huston's linker technology would not be expected to work for prodrug-activating enzyme

Even assuming, *arguendo*, that a person of ordinary skill in the art wanted to substitute the sFv fragment of Huston for the Fab fragment of the primary references, such a person would still have to analyze the antigenicity of different linkers. Huston states that the "immunogenicity [of linkers] may be negligible given the low sFv molecular weight and paucity of side chains in linkers." (*See* page 51, lines 34-35.) However, the size of a peptide structure is not a suitable measure of immunogenicity, and linkers are usually constructed in a way to be strongly exposed. Therefore, the immune system would be able to select antibodies against these exposed, albeit low weight, structures.

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In general, the linker architecture seems to have been considered as a limiting element of an sFv design in Huston. On page 52, lines 20-27 of the Huston article, it is stated that an improperly designed linker could cause conformational constraints or steric interference. On page 54, lines 1-2, the person of ordinary skill in the art learns that the linker should be hydrophilic to prevent intercalation within V domains. Much effort has been addressed to find out what linker length and type might be optimal. Table I summarizes linkers used for construction of different fusion proteins. A person of ordinary skill in the art, therefore, would have learned that fusion of a sFv molecule and an enzyme might be almost impossible to achieve when linker conditions are not appropriate. No teaching is given in Huston how to design an optimal linker under a given circumstance.

The Examiner has stated that Applicants' arguments regarding folding of the sFv construct "merely focus on some isolated reported cases of lowered binding affinities of sFv analogue and their fusion proteins and ignore the over all teaching that sFv analogues can be constructed with binding properties equivalent to those of the parent antibody." (Office Action, page 5.) The Examiner directs Applicants to statements in Huston on pages 87 and 68. Applicants respectfully point out that on page 55 of the Huston article, Huston references a general observation that deletion of not more than two (!) framework residues at the N terminus of V_H exhibited a very large alteration in binding affinity and specificity. Additionally, in the footnote on this page, Huston recognizes that certain fusions using a linker segment could result in the linker having an effect on the binding site properties.

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Thus, Huston provides evidence that the linker design is critical for fusion of sFv molecules to other proteins, as well as evidence that the linkers are often antigenic. Furthermore, Huston does not provide specific guidance as to which linkers would be expected to work for various protein fusion partners. A person of ordinary skill in the art faced with this evidence and lack of guidance would not have a reasonable expectation of success in designing an appropriate linker for fusing a sFv fragment to a prodrug-activating enzyme.

B. The Secondary References Do Not Cure the Deficiency in Huston

Applicants submit that the application of Bosslet et al. (EP 404,097) ("Bosslet"), Eaton (EP 392,745) ("Eaton"), Bagshawe et al. (WO 89/10140) ("Bagshawe"), and Goochee et al. (*Biotechnology*, 1991) ("Goochee") does not cure the deficiency discussed above with respect to Huston. Specifically, none of these references teach or suggest substituting a sFv for the Fab fragments of the primary references. Additionally, these references are not applicable to the claimed invention for the following reasons.

Bosslet teaches receptor molecules consisting of Fab fragments of at least two specificities by means of linkers. Bosslet does not teach or suggest the use of a single polypeptide antigen binding region, such as sFv fragments.

Eaton discloses certain prodrugs having a β-lactamase action that can be used in immunoconjugates. However, Eaton teaches the hydrolosis of a β-lactamate into various degradation products to form the active drug. The degradation products of

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Eaton are not precisely defined by structure, in contrast to the present inventive concept wherein the prodrug is a biologically inactive compound transformed into an active compound by the precise enzymatic removal of functional groups.

Ong relates to galactose conjugation of antibodies. As discussed above, the antibody structure does not provide a basis for creating a functional sFv construct since sFv constructs require the use of linkers which may cause possibly detrimental effects on folding, structure, and functionality of the overall molecule. Thus, Ong does not teach or suggest that the galactose conjugation is applicable to sFv constructs.

Bagshawe teaches the removal of residual antibody-enzyme from the blood after the conjugate has localized at tumor sites. According to Bagshawe's method, a first component *having no galactosyl residues* should be concentrated at the tumor sites. Thereafter, a second component having an antibody or fragment thereof and a number of covalently bonded galactose residues is introduced to clear the first component. Thus, Bagshawe is not applicable to the claimed invention wherein the conjugate itself may be modified by sugar residues.

Goochee teaches that biosynthesis of O-linked oligosaccharide structures in yeast is significantly different from mammalian O-glycosylation. On page 1352, right-hand column, Goochee specifically teaches against the use of yeast, insect, and plant cells as hosts for the production of human therapeutic glycolproteins. Accordingly, Goochee teaches away from the instantly claimed invention.

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VI. Conclusion

Applicants respectfully requests that this Amendment under 37 C.F.R. § 1.116 be entered by the Examiner, placing claims 1-13 and 25-33 in condition for allowance. Applicants submit that the proposed amendments of claims 1, 2, 3, 29, and 30 do not raise new issues or necessitate the undertaking of any additional search of the art by the Examiner, since all of the elements and their relationships claimed were either earlier claimed or inherent in the claims as examined. Therefore, this Amendment should allow for immediate action by the Examiner.

Finally, Applicants submit that the entry of the amendment would place the application in better form for appeal, should the Examiner dispute the patentability of the pending claims.

In view of the foregoing remarks, Applicants submit that this claimed invention, as amended, is neither anticipated nor rendered obvious in view of the prior art references cited against this application. Applicants therefore request the entry of this Amendment, the Examiner's reconsideration and reexamination of the application, and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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Dated: November 7, 2000

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